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Method for the Confirmation of Chlorophenols in Human Urine by LC with an Electrochemical Detector

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JOURNAL ARTICLE

Method for the Confirmation of Chlorophenols in Human Urine by LC with an Electrochemical Detector

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Abstract

A method is described for the confirmation of chlorophenols in human urine. A hydrolyzed urine sample is analyzed by both gas chromatography (GC), and liquid chromatography (LC) with electrochemical detection and the results compared. A discussion of these results, including factors affecting separation, detection, and confirmation of chlorophenols in human urine, is included.

Introduction

Chlorophenols represent a group of pollutants commonly found in the environment. These compounds arise from degradation or metabolism of pesticides, chlorination of water supplies, and direct introduction from industrial sources (1-3). They may appear in the urine of humans as a result of direct exposure to environmental sources or from exposure to pesticides or other compounds that metabolically give rise to chlorinated phenols.

The advent of liquid chromatography (LC) and electrochemical (EC) detection (4,5) has allowed for the development of sensitive analytical methods and confirmation techniques for the determination of phenolic compounds without derivatization. Since many of the reagents used for derivatizing phenols (6) are carcinogenic, toxic, or explosive and derivatization adds a step to the analysis which increases the chance for error, methods that do not require derivatization are highly desirable. A method using LC and EC detection (LC/EC) has been reported for the analysis of thirteen chlorinated phenols in water (7). In order to adapt the reported method from the analysis of water to the analysis of urine, additional cleanup of sample extracts was needed because urine contains many interfering electroactive compounds. Also, in order to determine all of the nineteen possible chlorophenol isomers, the resolution of chlorophenols as reported previously (7) required improvement.

A method for the extraction, cleanup, and analysis of chlorophenols in urine by GC has been developed by Edgerton, et al. (8). Using these sample preparation techniques, a variety of human urine samples were analyzed with the LC/EC system and the results compared to those obtained by the GC method. A discussion of these results along with factors affecting separation, detection, and confirmation of chlorophenols in human urine is included in the text.

Experimental*

The LC system consisted of a Tracor Model 995 (Austin, Texas) isochromatographic pump with a Model CV-6-UHPa-HC Valco (Houston, Texas) valve and a Bioanalytical Systems (West LaFayette, Indiana) Model LC-2A EC detector. The EC detector was operated at an oxidation potential of +1.1 V. Wax-impregnated carbon paste (Bioanalytical Systems) or glassy carbon electrodes were used. Zorbax® C8 columns of 15 or 25 cm lengths were used throughout the study. Two different mobile phases were required because gradient elution cannot be used with the EC detector.

The mobile phase used for the early eluting phenols was 46% 0.05 *M*, pH 4.0, phosphate buffer, 40% methanol, and 14% acetonitrile. The mobile phase used for the later eluting phenols was 50% phosphate buffer and 50% acetonitrile. All solvents were chromatographic grade and were filtered through 0.4 μ filters before use. All phenol standards were obtained from Aldrich Chemical Company (Milwaukee, Wisconsin).

Hydrolysis and Cleanup of Urine for Gas Chromatographic Analysis

Void urine samples were obtained from volunteer subjects and were prepared for analysis by the method of Edgerton, et al. (8). Briefly, a 4 ml aliquot of urine was placed in a 15 mm x

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125 mm screw-cap culture tube, 1 ml concentrated hydrochloric acid was added, and the tube was capped and heated in a boiling water bath for 1 hr. The hydrolyzed urine was transferred to an XAD-4 macroreticular resin column. After several aqueous washes, the chlorinated phenols were eluted with 10% 2-propanol in hexane. This fraction, which contained acidic compounds, was concentrated to an appropriate volume and analyzed by GC using the electron capture detector (EC/GC).

Cleanup of Urine for LC Confirmation

After EC/GC analysis the propanol/hexane fraction was partitioned with a volume of 0.1 *M* NaOH equal to the original volume of the urine sample. The aqueous layer was transferred to a 13 ml graduated centrifuge tube and acidified with concentrated H_3PO_4 to a pH of 3 to 4, as determined with pHydrion® paper. A 100 µl aliquot from the aqueous phase was then injected into the liquid chromatograph.

Results and Discussion

The phenols used in this study and some of the environmental origins are listed in Table I. A comparison of the results from LC and EC/GC is shown in Table II. In general, there is agreement between the results obtained by the two methods for pentachlorophenol, 2,4,5-trichlorophenol, and 2,4- or 2,5-dichlorophenol (the latter two isomers being separated by LC but not by EC/GC). The EC/GC analysis appears to give false positives for 2,6- and 3,5-dichlorophenol while LC/EC gives false positives for 2,3-dichlorophenol and 2,4,6-trichlorophenol. These data support the complementary use of both EC/GC and LC/EC for identification and confirmation of chlorophenols in human urine.

The EC/GC technique provides lower limits of detection for all except the monochlorophenols, which are relatively nonresponsive to electron capture detection. However, the limit of detection with LC could be improved if the sample were concentrated and some of the interferences removed.

Figure 1 shows representative LC/EC chromatograms of chlorophenol standards and a human urine sample carried through the procedure using the mobile phase for early eluting phenols. As can be seen, there are numerous unidentified peaks in addition to the chlorophenol standards. The elution times for several of these extraneous compounds were found to correspond to phenolic metabolites from common pesticides such as carbaryl.

Table I. Possible Origins of Various Chlorinated Phenols

Metabolite	Origin	Type Pesticide
2-Chlorophenol	2,4-D	Herbicide
3-Chlorophenol	PCP	Fungicide
4-Chlorophenol	Chloroxuron	Herbicide
2,6-Dichlorophenol	Lindane	Insecticide
2,4-Dichlorophenol	VC-13	Insecticide
	m-Dichlorobenzene	Fumigant
	2,4-D	Herbicide
2,3-Dichlorophenol	Lindane	Insecticide
	o-Dichlorobenzene	Fumigant
2,5-Dichlorophenol	Lindane	Insecticide
	p-Dichlorobenzene	
	2,4,5-T	Herbicide
3,4-Dichlorophenol	PCP	Fungicide
	o-Dichlorobenzene	Fumigant
	Diuron	Herbicide
3,5-Dichlorophenol	Lindane	Insecticide
	PCP	Fungicide
2,3,4-Trichlorophenol	Lindane	Insecticide
2,3,5-Trichlorophenol	Lindane	Insecticide
	PCP	Fungicide
2,3,6-Trichlorophenol	Lindane	Insecticide
2,4,5-Trichlorophenol	Ronnel	Insecticide
	Tetrachlorvinphos	Insecticide
	Erbon	Herbicide
	2,4,5-T	Herbicide
	HCB	Fungicide
2,4,6-Trichlorophenol	Lindane	Insecticide
3,4,5-Trichlorophenol	Lindane	Insecticide
2,3,5,6-Tetrachlorophenol	HCB	Fungicide
	PCP	
2,3,4,6-Tetrachlorophenol	PCP (impurity)	Fungicide
	Lindane	Insecticide
2,3,4,5-Tetrachlorophenol	PCP	Fungicide
	Lindane	Insecticide
	HCB	Fungicide
Pentachlorophenol	PCP	Fungicide
	Lindane	Insecticide
	HCB	Fungicide
	PCNB	Fungicide

Table II. Comparison of Chlorophenol Levels Found in Human Urine by LC/EC and EC/GC

	Chlorophenols (results in ppb)																	
	2,6-Di-		2,5-Di-	2,4-Di-	2,5- or 2,4-Di-	2,3-Di-		2,3,6-Tri-		3,5-Di-		2,4,5-Tri-		2,4,6-Tri-		2,3,4,6-Tetra-		Penta-	
Sample	LC	GC	LC	LC	GC	LC	GC	LC	GC	LC	GC	LC	GC	LC	GC	LC	GC	LC	GC
MDS 101	<2	81	56	<6	45	51	<1	<10	<1	20	42	<6	2	40	<1	<10	8	10	16
102	3	21	130	<6	117	130	<1	<10	<1	<20	21	<6	4	<10	<1	<10	6	10	11
103	6	31	18	<6	15	<10	<1	<10	<1	<20	9	<6	3	200	<1	<10	6	6	9
104	<2	69	<6	<6	13	<10	<1	<10	<1	<20	6	<6	2	25	<1	<10	4	9	3
105	10	40	160	<6	132	<10	<1	<10	<1	<20	2	<6	5	55	<1	<10	7	7	6
106	<2	36	27	<6	13	<10	<1	33	<1	<20	11	<6	<1	65	<1	<10	3	7	5
108	<2	31	200	<6	156	<10	<1	20	<1	<20	18	30	37	100	<1	<10	9	<5	9
109	<2	50	<6	<6	9	<10	<1	<10	<1	<20	36	<6	4	75	<1	<10	7	<5	14
110	<2	66	250	<6	179	<10	<1	<10	<1	<20	12	<6	<1	100	<1	<10	6	16	13
111	<2	99	20	<6	25	<10	<1	<10	<1	<20	30	<6	<1	100	<1	<10	3	<5	6

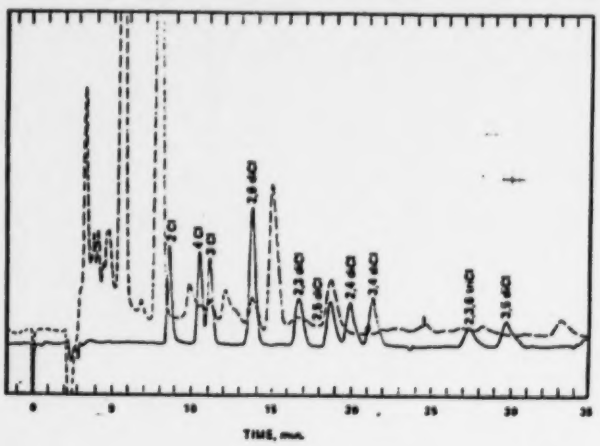

Figure 1. Chromatograms of early eluting chlorophenols. The mobile phase consisted of 45% 0.05 M phosphate buffer, pH 4.0, 40% methanol, and 14% acetonitrile. The dotted line represents an injection of 100 µl of urine carried through the procedure. The solid line is a chromatogram of standards representing approximately 10 ng of each chlorophenol shown.

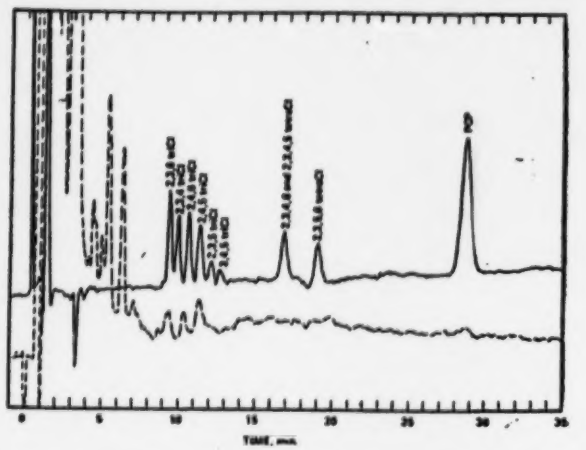

Figure 2. Chromatograms of the later eluting phenols. The mobile phase consisted of 50% acetonitrile and 50% phosphate buffer. The dotted line represents an injection of 100 µl of urine carried through the procedure. The solid line is a chromatogram of standards representing approximately 10 ng of each chlorophenol, except pentachlorophenol which represents approximately 30 ng.

Figure 2 shows chromatograms of chlorophenol standards and a human urine sample carried through the procedure using the mobile phase for late eluting phenols. As can be seen, the responses of the tetrachlorophenols are lower than the other chlorophenols, with the exception of 3,5-dichloro- and 2,3,5-trichlorophenol. This is apparently due to a stabilizing effect of the *meta-meta* substitution which makes oxidation more difficult.

The recovery of chlorophenols through the entire procedure is equivalent to that reported by Edgerton, et al. (8). The only step added to the GC procedure is the organic/base partition prior to LC analysis. It has been shown that chlorophenols are quantitatively recovered through this portion of the method.

The detector used in this study is sensitive to only those compounds which can be oxidized at or below the applied potential used (+1.1 V). Cleanup of the sample eliminates most of the neutral and basic compounds that might be in the urine. Of the remaining acidic compounds, the majority of those giving a response are probably phenols. Some acids, such as salicylic acid and vanillic acid, produce responses using the electrochemical detector, since they also have aromatic hydroxyl substituents that can be oxidized. However, carboxylic acids in general are not detected electrochemically (4,5).

Since the number of phenols that can occur in urine is almost infinite and the ECD will detect almost all phenols, further study is needed to explore methodology that will allow separation of phenols into smaller groups which can then be analyzed by LC/EC. Depending upon the response characteristics of the specific phenol, the LC/EC confirmation method presented here can detect levels of all nineteen chlorophenols in the low parts-per-billion range. With the use of appropriate standards, it can be used for identification and confirmation of other phenols that occur in urine. In fact, it is believed that most of the interferences found in the urine using this method are from dietary origins or cresols and other phenols that result from the metabolism of carbamate pesticides, such as carbaryl and mexacarbate. These phenolic compounds coelute with peaks that commonly occur in the urine samples studied. This confirmation method shows promise for use as an index of exposure for many of the phenols that result from environmental exposure. However, additional work to provide for better cleanup and/or separation of phenols prior to LC/EC determination would be needed before this confirmation method could be used as a method for analysis of chlorophenols in urine.

Conclusion

Methodology has been presented for confirmation of chlorophenols in human urine. The method utilizes LC with electrochemical detection and does not require a derivatization step. This confirmation method should be useful in measuring indexes of human exposure to compounds that give rise to chlorophenol residues in urine. The method is sensitive to chlorophenols at the low parts-per-billion range as they are encountered in most general population samples.

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